methods for arsenic speciation, Amoco selected an analytical method following an exhaustive review of peer-reviewed literature. A quality assurance/quality control (QA/QC) program was developed in order to have confidence in the ability of the analytical methods to speciate and detect arsenic.

DESCRIPTION OF TERMS

There are two forms of arsenic: organic and inorganic. Organic arsenic was not measured in the treated effluent, as discussed in Section 4, therefore, it is not discussed in this report. The two species of inorganic arsenic discussed in this report are trivalent arsenic or arsenic(III), and pentavalent arsenic or arsenic(V). "Total arsenic", as used in this report, is equivalent to the term "total recoverable arsenic", which is the sum of all forms and species of arsenic.

SECTION 2

WATER QUALITY CRITERIA FOR ARSENIC

DEVELOPMENT OF USEPA WATER QUALITY CRITERIA FOR ARSENIC

Federal water quality criteria for arsenic were first developed in *Ambient Water Quality for Arsenic* (USEPA, October, 1980). These criteria were also included in *Quality Criteria for Water 1986* (USEPA, May, 1986 and updated September 2, 1986) also known as the Gold Book. Criteria were adopted by USEPA for the following conditions:

- freshwater acute;
- freshwater chronic;
- fish ingestion; and
- fish and water ingestion.

Table 2-1 presents the water quality criteria recommended by the USEPA in 1986 and adopted by Indiana on March 3, 1990. The USEPA aquatic life water quality criteria for arsenic(III) were derived based on the standard statistical analysis of laboratory bioassay test data for arsenic(III). The human health criteria reported concentrations for arsenic(III) of 0.0022 μg/L for the consumption of fish and water and 0.0175 μg/L for consumption of fish only (at a risk level of 10^{-6}).

INDIANA WATER QUALITY CRITERIA FOR ARSENIC(III)

After the USEPA had identified, in 1986, trivalent arsenic as the species of most concern, Indiana included arsenic(III) on the list of constituents having acute aquatic or continuous chronic criteria. The Indiana Water Quality Standards (IWQS) for arsenic(III) are presented in Table 2-2. Indiana adopted the two Gold Book human health criteria for:

1. Fish Ingestion (consumption), and
2. Fish and Water Ingestion (consumption).

Indiana's rule applies the two Gold Book human health criteria at two different points. The fish consumption criterion is applied outside the mixing zone and the fish and water consumption criterion is applied at point of water intake. No criteria were adopted by Indiana for arsenic(V) or total arsenic. Based on a review of water quality standards for other Great Lakes states, no other state regulates arsenic(III) based on the USEPA Gold Book human health criteria for consumption of fish. Only Indiana and Pennsylvania have the arsenic(III) standard for the USEPA Gold Book human health criteria based on the consumption of fish and water. Pennsylvania, like Indiana, applies this particular criterion at a point of water intake. No other Great Lakes state regulates arsenic(III) based on the USEPA Gold Book human health criterion for consumption of fish and water.

Amoco's existing treated effluent meets the Gold Book aquatic life criteria for arsenic(III), at end of pipe, as measured by total arsenic. However, the total arsenic amount measured in the treated effluent is greater than the arsenic(III) Gold Book human health criteria for fish ingestion. Due to the nature of the effluent, Amoco was convinced that arsenic(III), the regulated species of arsenic, did not exist in the treated effluent. To confirm this conviction, Amoco analytically determined that its total arsenic was not arsenic(III).

TABLE D2–1. USEPA WATER QUALITY CRITERIA FOR ARSENIC (a)

CONSTITUENT	AQUATIC LIFE		HUMAN HEALTH	
	FRESHWATER ACUTE (µg/L)	FRESHWATER CHRONIC (µg/L)	FISH INGESTION (µg/L)	WATER AND FISH INGESTION (µg/L)
Arsenic (III) (b)	360	190	0.0175	0.0022
Arsenic (V) (c)	850	48	None	None

NOTES:

(a) Sources: "Ambient Water Quality for Arsenic", USEPA, October, 1980.
"Quality Criteria for Water 1986", USEPA, May, 1986, plus first update on September 2, 1986.

(b) Numeric criteria derived based on standard methods.

(c) Lowest observable effect level (LOEL).

NA – Not applicable

TABLE D2–2. INDIANA WATER QUALITY CRITERIA FOR ARSENIC (a)

CONSTITUENT	ACUTE AQUATIC CONCENTRATION (AAC) (maximum) (µg/L)	CONTINUOUS CHRONIC CONCENTRATION		
		OUTSIDE OF MIXING ZONE		POINT OF WATER INTAKE (µg/L)
		CHRONIC AQUATIC (µg/L)	HUMAN HEALTH (µg/L)	
Arsenic (III)	360	190	0.175	0.022

NOTES:
(a) Source: 372 IAC 2–1–6 (a) Table 1.

SECTION 3

ARSENIC CHEMISTRY

ENVIRONMENTAL CHEMISTRY OF ARSENIC

Arsenic is encountered in the natural environment in both inorganic and organic forms. The predominant valence states for arsenic in both organic and inorganic forms are arsenic(III) and arsenic(V). As discussed in Section 2.0, arsenic(III) is the more toxic form, both to human health and aquatic life. It appears as arsenious acid or its anion in most aquatic systems. Arsenic(V), however, is the predominant form of the metal under normal conditions found in surface waters. It appears in solution as an anion of arsenic acid. The dissociation of arsenic(V) can be written as follows:

$$H_3AsO_4 = H^+ + H_2AsO_4^- \qquad pKa = 2.2$$

$$H_2AsO_4^- = H^+ + HAsO_4^{2-} \qquad pKa = 6.98$$

$$HAsO_4^{2-} = H^+ + AsO_4^{3-} \qquad pKa = 11.5$$

The dissociation of arsenic(III) can be written as:

$$As(OH)_3 = As(OH)_2^- + H^+ \qquad pKa = 9.2$$

Therefore, in the pH range of 6 to 8, arsenic(V) occurs as either single- or double-charged anion, whereas arsenic(III) would be present as arsenious acid. As a result of the arsenic species interconvertability, arsenic(V) predominates under the aerobic conditions that almost always exist in surface waters. Conversely, as indicated in *Arsenic(III) Oxidation and Removal From Drinking Water,* (USEPA, February 1986) arsenic (III) is the predominant species in anaerobic groundwaters where conditions of low pH (less than pH 6.0), low oxidation-reduction potential (E_h is negative), and low dissolved oxygen (below 2 to 4 mg/L) exist. Conversion of arsenic(III) to arsenic(V) is rapid under any oxidizing conditions. These oxidizing conditions exist in refinery production processes, aerobic wastewater treatment units, and

Each of the approved methods require digestion prior to analysis to solubilize suspended material and destroy organic-metal complexes. Each of the test procedures outlined in *Standard Methods for the Analysis of Water and Waste*, 18th Edition (Standard Methods) (see Appendix 1) is briefly described below. Although some of the listed methods include test procedures for arsenic (III) in addition to total arsenic, only the total arsenic test method is approved by the referenced USEPA regulations.

Atomic Absorption Gaseous Hydride Method

This method determines total arsenic by converting arsenous acid, i.e. arsenic(III), to its volatile hydride using sodium borohydride. The hydride is purged continuously by argon or nitrogen into an atomic absorption atomizer. Determination of total arsenic requires the oxidation of organic and inorganic forms of arsenic to arsenic(V) and then reducing them to arsenic(III) before initiating the sodium borohydride reaction. Although some of the listed methods include test procedures for arsenic(III) in addition to total arsenic, only total arsenic is approved by the referenced USEPA regulations.

Atomic Absorption Furnace Technique

This method injects a volume of pretreated sample, equal to the calibration volume, into the graphite furnace. The sample is then dried, charred, and atomized. The average absorbance value or peak area is compared to the calibration curve to determine concentration. This method is suitable for determining micro-quantities of arsenic.

TABLE D3-1. STANDARD REDUCTION POTENTIALS FOR VARIOUS ARSENIC SPECIES

Reaction	Potential (Volts)
$As + 3H^+ + 3e^- \rightarrow AsH_3$	-0.54
$As_2O_3 + 6H^+ + 6e^- \rightarrow 2As + 3H_2O$	0.234
$HAsO_2 + 3H^+ + 3e^- \rightarrow As + 2H_2O$	0.2475
$AsO_2^- + 2H_2O + 3e^- \rightarrow As + 4OH^-$	-0.68
$H_3AsO_4^{-3} + 2H^+ + 2e^- \rightarrow HAsO_2 + 2H_2O$(if HCl)	0.58
$AsO_4^{-3} + 2H_2O + 2e^- \rightarrow AsO_2^- + 4OH^-$	-0.71
$AsO_4^{-3} + 2H_2O + 2e^- \rightarrow AsO_2^- + 4OH^-$ (if NaOH)	0.08

TABLE D3–2. APPROVED TOTAL ARSENIC ANALYTICAL PROCEDURES (a)

PARAMETER UNITS AND METHOD	REFERENCE (Method No. or Page)			
	EPA (b,c)	STD. METHODS 18th Ed.	ASTM	USGS (d)
Arsenic – Total (e) mg/L				
Digestion (e) followed by	206.5			
AA gaseous hydride	206.3	3114 B 4.d	D2972–88(B)	I–3062–85
AA furnace	206.2	3113 B	D2972–88(C)	
ICP/AES (c)	200.7 (f)	3120 B		
Colorimetric (SDDC)	206.4	3500–As C	D2972–88(A)	I–3060–85

NOTES:

(a) EPA Regulations on Test Procedures for the Analysis of Pollutants (40 CFR 136)

(b) "Methods for Chemical Analysis of Water and Wastes", Environmental Monitoring Systems Laboratory–Cincinnati (EMSL–CI), EPA–600/4–79–020, Revised March 1983 and 1979 where applicable.

(c) "Closed Vessel Microwave Digestion of Wastewater Samples for Determination of Metals", CEM Corporation, P.O. Box 200, Matthews, NC 28106–0200, April 16, 1992. Available from the CEM Corporation.

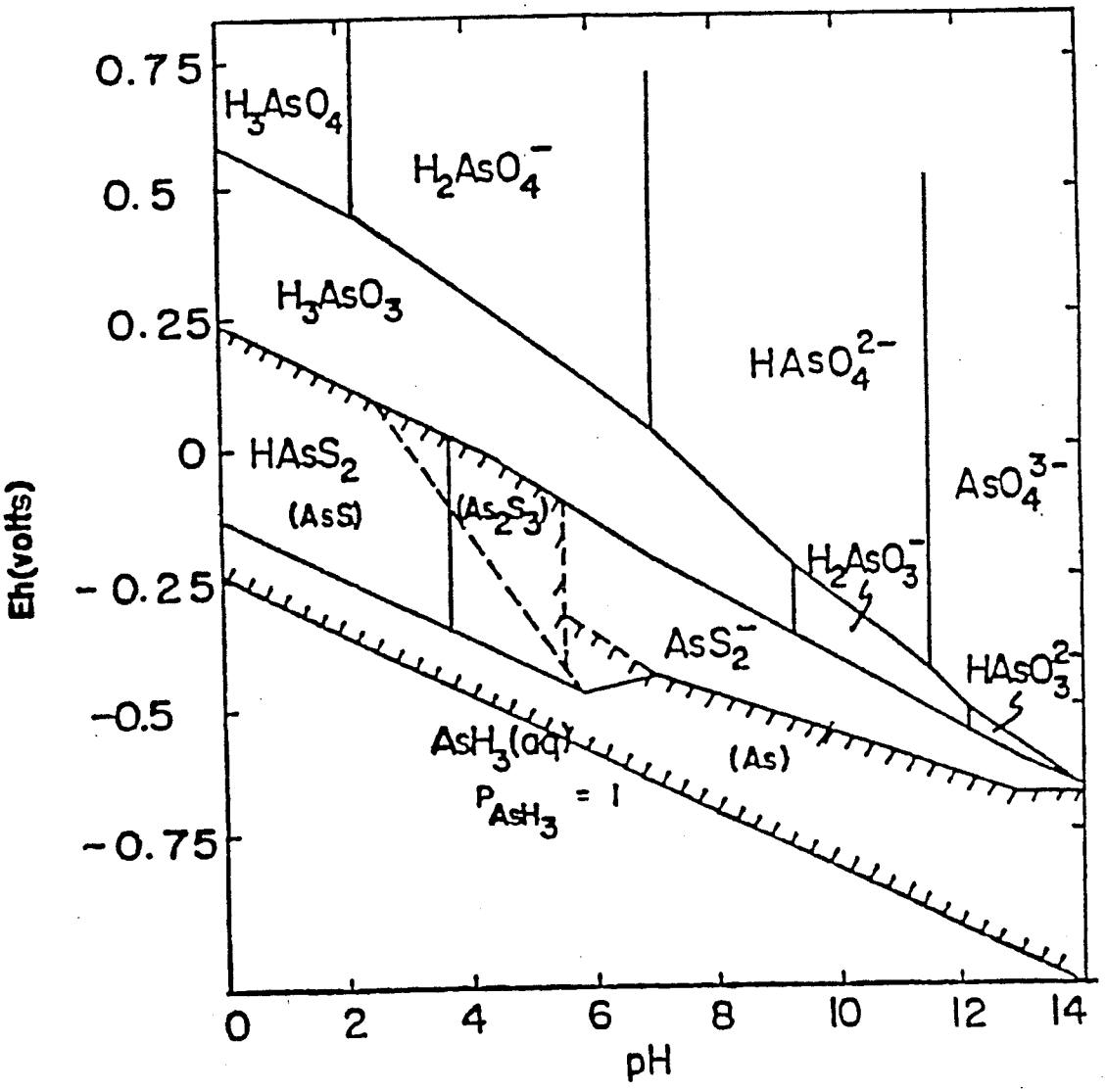
(d) Fishman, M.J., et al, "Methods for Analysis of Inorganic Substances in Water and Fluvial Sediments," U.S. Department of the Interior, Techniques of Water–Resource Investigations of the U.S. Geological Survey, Denver, CO, Revised 1989, unless otherwise stated.

(e) For the determination of total metals the sample is not filtered before processing. A digestion procedure is required to solublize suspended material and to destroy possible organicmetal complexes. Two digestion procedures are given in "Methods for Chemical Analysis of Water and Wastes, 1979 and 1983". One (section 4.1.3), is a vigorous digestion using nitric acid. A less vigorous digestion using nitric and hydrochloric acids (section 4.1.4) is preferred; however, the analyst should be cautioned that this mild digestion may not suffice for all sample types. Particularly, if a colormetric procedure is to be employed, it is necessary to ensure that all organo–matllic bonds be broken so that the metal is in a reactive state.

Dissolved metals are defined as those constituents which will pass through a 0.45 micron membrane filter. The referenced procedure for total metals must be followed. Sample digestion of the filtrate for dissolved metals may be omitted for AA and ICP analysis, provided the sample solution to be analyzed meets the following criteria.

a. Has a low COD (<20)
b. Is visibly transparent with a turbidity measurement of 1 NTU or less,
c. Is colorless with no perceptible odor, and
d. is of one liquid phase and free of particulate or suspended matter following acidification.

(f) The full text of Method 200.7, "Inductively Coupled Plasma Atomic Emission Spectrometric Method for Trace Element Analysis of Water and Wastes," is given at Appendix C of 40 CFR 136.



Source:- *A Review of the Arsenic Cycle in Natural Waters.* **Water Research.** Pergamon Press: Great Britain, 1972. Vol. 6, pp. 1259-1274

FIGURE 3-1. E_h-pH DIAGRAM FOR ARSENIC AT 25° C AND 1 Atm. PRESSURE

SECTION 4

DEVELOPMENT OF ARSENIC SPECIATION STUDY

ANALYTICAL METHODS FOR ARSENIC SPECIATION

Arsenic speciation methods have been presented in peer-reviewed literature. A bibliography of analytical procedures reported in the literature is presented in Appendix 2. These peer-reviewed published methods were reviewed for applicability to Amoco's treated effluent. After extensive review, three methods were selected for further analysis:

- SDDC Method;
- APDC-Graphite Furnace Atomic Absorption method; and,
- HPLC-Graphite Furnace Atomic Absorption method.

As discussed below, only the HPLC-Graphite Furnace Atomic Absorption method successfully speciated arsenic in the Amoco treated effluent.

The SDDC method listed in Section 3.2 of Standard Methods is a method for analyzing trivalent arsenic (see Appendix 1). This method involves using sodium borohydride to reduce arsenic(III) to arsine gas, scrubbing this gas with lead acetate solution, and then absorbing it in silver diethylithic carbamate and morphine dissolved in chloroform. The level of arsenic(III) is then determined colormetrically. Amoco contacted laboratories known to perform arsenic speciation analysis, none of these laboratories were capable of performing this analysis.

A procedure by Montgomery Laboratories, of Pasadena, California involves reducing inorganic arsenic to arsenic(III), complexing with ammonium pyrrolidine dithiocarbamate (APDC), partitioning from the water sample onto a C_8 disk, eluting from the disk with acidic methanol, evaporating to near dryness, diluting with 1 percent HNO_3, and analyzing by Graphite Furnace Atomic Absorption (GFAA). For the determination of arsenic(III) only, the reduction step is omitted and arsenic(III) and arsenic(V) can be determined sequentially. Montgomery Laboratories and Amoco determined that this complexation method could not

reliably speciate arsenic(III) and arsenic(V) in Amoco's treated effluent. This determination was based on a review of internal QA/QC data, results of Amoco field QA/QC, and Amoco's spike samples.

An arsenic speciation method developed in 1980 by the U.S. Department of Agriculture (USDA)[1] and performed by EPL Bio-Analytical, Inc., (EPL) of Harristown, Illinois (provided in Appendix 4) utilizes High Performance Liquid Chromatography (HPLC) and Graphite Furnace Atomic Absorption (GFAA). With this method water samples are filtered and concentrated as necessary for analysis. Arsenic speciation analysis is performed using anion exchange HPLC with GFAA detection. Filtered samples are also analyzed by GFAA with a Zeeman background correction to obtain soluble arsenic values. Note that based on the total versus dissolved metals ratio study presented as Volume I Form 2C Item V Footnote C, arsenic is present in the effluent as a soluble metal. EPL also analyzes the sample for total arsenic using USEPA Method 206.2.

The HPLC/GFAA method involves two widely accepted analytical instruments. The prepared and extracted effluent sample is injected into the HPLC column. The different species of arsenic travel through the column at different rates based on their interaction with the mobile phase and the columns stationary phase. The HPLC chromatographically separates the arsenic species generating a continuous column eluant. To detect the constituents in the column eluant a GFAA is used. Because the GFAA is not a continuous flow instrument, small portions of the HPLC column eluant are collected periodically (e.g., 10 aliquots over 5 minutes)

---

[1] JAOAC, Vol 63, No 4, 1980 pg 742-746.
JAOAC, Vol 63, No 3, 1980, pg 523-528.

over time of the HPLC run. The GFAA detects the presence of arsenic in the collected aliquots that are collected over time. Each aliquot is specific to the time it takes a species to pass through the HPLC column. If the species is present in an aliquot, the GFAA detector, calibrated for arsenic, produces a quantitatively proportional change in an electrical signal that is measured on a recorder chart. This change in the electrical signal is normally seen as a deflection upwards from a baseline and is referred to as the "peak height" for the compound. The time at which the peak height appears is the retention time indicative of the arsenic species and the peak height is proportional to its concentration.

The relative fractions of the species measured by HPLC/GFAA are expressed as normalized peak heights for the individual arsenic species. The normalized peak heights for the arsenic species contained in a sample are based on:

- the cumulative peak height for arsenic(III) and arsenic(V) standards analyzed by HPLC/GFAA;
- the cumulative peak height at the applicable arsenic species retention time, as expressed by the GFAA detector:
- the response factor for each arsenic standard calculated by dividing the cumulative peak height by the standard concentration; then,
- calculating the normalized peak height for the arsenic species detected in the sample by dividing the cumulative peak height by the standard response factor.

These normalized peak heights can be translated to percent (%) relative amounts of each arsenic species detected in the sample. The percent relative amounts for each species is applied to the quantitation of total arsenic by USEPA Method 206.2 (GFAA) and can be used to develop an approximate quantity of arsenic(III) and arsenic(V) in a sample. This type of quantitation is used due to the fact that the HPLC/GFAA measurement method is

discontinuous. The discontinuous analysis arises because it is not possible to have a direct coupling of the HPLC to the GFAA; hence, the time required to determine response of the HPLC eluting peak is a function of the GFAA recycle rate, normally 45 seconds. This means that the HPLC column eluant that elutes between GFAA cycle time required for analysis is generally not analyzed; hence, the quantitation accuracy is not equivalent to the accuracy achieved by USEPA Method 206.2 when applied to total arsenic.

The HPLC/GFAA detection limit for aqueous samples has been conservatively established over a several year period by EPL of 100 μg/L. For this arsenic speciation study, samples are concentrated 20 times prior to analyses, giving a detection limit of 5 μg/L. EPL asserts that this method can reliably speciate arsenic(III) to a detection limit of 3 μg/L. USEPA Method 206.2 method detection limit for arsenic is 2 μg/L.

Given that the EPL HPLC/GFAA method was the only method that could reliably speciate the arsenic in Amoco's effluent, this method was used to characterize the arsenic in the Outfall 001 effluent.

SAMPLING AND ANALYSIS PROGRAM

A sampling and analysis program was developed to collect sufficient data to characterize the species of the arsenic present in the Outfall 001 effluent discharge. The laboratories selected to participate in the study were:

- Laboratory 1 - Amoco Research and Development Laboratory, Naperville, Illinois;
- Laboratory 2 - EPL Bio-Analytical, Inc., Harristown, Illinois; and,

Laboratory 1 was selected to provide total arsenic analyses only. This is the same laboratory that analyzed the samples to characterize the Outfall 001 discharge for total arsenic for reporting on the Form 2C of the NPDES Permit Application.

It should be noted that dissolved (soluble) arsenic was quantified, and the total arsenic present in the effluent was primarily in the soluble form. Laboratory 2 was selected to provide total arsenic and inorganic arsenic speciation analyses. No organic arsenic species were detected in the samples. A summary of the sampling and analysis program is provided in Table 4-1.

TABLE D4-1. ARSENIC SPECIATION SAMPLING SCHEDULE for NPDES PERMIT APPLICATION

WEEK	SAMPLE NAME	VOLUME	SAMPLE HANDLING	LABORATORY (a)
1	1-N-0	250 mL	Ultrapure HNO3, pH < 2, 4°C	Amoco Naperville R&D
	1-B-0	1000 mL	Collect at 4°C; freeze, ship on dry ice	EPL Bio-analytical
2	2-N-0	250 mL	Ultrapure HNO3, pH < 2, 4°C	Amoco Naperville R&D
	2-N-1	250 mL	Duplicate sample, second aliquot poured from 24-hr composite jug; ultrapure HNO3, pH < 2, 4°C	Amoco Naperville R&D
	2-N-2	1000 mL	Collect at 4°C; add 1 drop Na Arsenite, add 5 drops Arsenic Acid solution, acid pH < 2, 4°C	Amoco Naperville R&D
	2-N-3	1000 mL	De-ionized water; acid pH < 2, 4°C (Field Blank)	Amoco Naperville R&D
	2-B-0	1000 mL	Collect at 4°C; freeze, ship on dry ice	EPL Bio-analytical
	2-B-1	1000 mL	Collect at 4°C; Duplicate sample, freeze, ship on dry ice	EPL Bio-analytical
	2-B-2	1000 mL	Collect at 4°C; add 1 drop Na Arsenite, add 5 drops Arsenic Acid Solution, freeze; ship on dry ice	EPL Bio-analytical
	2-B-3	1000 mL	De-ionized water; freeze at same time composite sample prep; ship on dry ice (Field Blank)	EPL Bio-analytical
3	3-N-0	250 mL	Ultrapure HNO3, pH < 2, 4°C	Amoco Naperville R&D
	3-B-0	1000 mL	Collect at 4°C; freeze, ship on dry ice	EPL Bio-analytical
4	4-N-0	250 mL	Ultrapure HNO3, pH < 2, 4°C	Amoco Naperville R&D
	4-B-0	1000 mL	Collect at 4°C; freeze, ship on dry ice	EPL Bio-analytical
5	5-N-0	250 mL	Ultrapure HNO3, pH < 2, 4°C	Amoco Naperville R&D
	5-N-1	250 mL	Duplicate sample, second aliquot poured from 24-hr composite jug; ultrapure HNO3, pH < 2, 4°C	Amoco Naperville R&D
	5-N-2	1000 mL	Collect at 4°C; add 1 drop Na Arsenite, add 5 drops Arsenic Acid solution, acid pH < 2, 4°C	Amoco Naperville R&D
	5-N-3	1000 mL	De-ionized water; acid pH < 2, 4°C (Field Blank)	Amoco Naperville R&D
	5-B-0	1000 mL	Collect at 4°C; freeze, ship on dry ice	EPL Bio-analytical
	5-B-1	1000 mL	Collect at 4°C; Duplicate sample, freeze, ship on dry ice	EPL Bio-analytical
	5-B-2	1000 mL	Collect at 4°C; add 1 drop Na Arsenite, add 5 drops Arsenic Acid Solution, freeze; ship on dry ice	EPL Bio-analytical
	5-B-3	1000 mL	De-ionized water; freeze at same time composite sample prep; ship on dry ice (Field Blank)	EPL Bio-analytical
6	6-N-0	250 mL	Ultrapure HNO3, pH < 2, 4°C	Amoco Naperville R&D
	6-B-0	1000 mL	Collect at 4°C; freeze, ship on dry ice	EPL Bio-analytical
7	7-N-0	250 mL	Ultrapure HNO3, pH < 2, 4°C	Amoco Naperville R&D
	7-N-1	250 mL	Duplicate sample, second aliquot poured from 24-hr composite jug; ultrapure HNO3, pH < 2, 4°C	Amoco Naperville R&D
	7-N-2	1000 mL	Collect at 4°C; add 1 drop Na Arsenite, add 5 drops Arsenic Acid solution, acid pH < 2, 4°C	Amoco Naperville R&D
	7-N-3	1000 mL	De-ionized water; acid pH < 2, 4°C (Field Blank)	Amoco Naperville R&D
	7-B-0	1000 mL	Collect at 4°C; freeze, ship on dry ice	EPL Bio-analytical
	7-B-1	1000 mL	Collect at 4°C; Duplicate sample, freeze, ship on dry ice	EPL Bio-analytical
	7-B-2	1000 mL	Collect at 4°C; add 1 drop Na Arsenite, add 5 drops Arsenic Acid Solution, freeze; ship on dry ice	EPL Bio-analytical
	7-B-3	1000 mL	De-ionized water; freeze at same time composite sample prep; ship on dry ice (Field Blank)	EPL Bio-analytical

NOTES:

(a) Amoco Naperville R&D laboratory, in Naperville, Illinois, analyzes total arsenic.
EPL Bio-analytical, Inc., in Harristown, Illinois, analyzes total arsenic and arsenic speciation.

(b) Plastic containers were used by both laboratories.

SECTION 5

RESULTS OF ARSENIC SPECIATION STUDY

The analytical results for the arsenic speciation study are discussed in this section. Complete analytical reports are included in Appendix 4.

ARSENIC SPECIATION RESULTS

The arsenic speciation results presented in this section were determined using the EPL Bio-Analytical, Inc. (EPL) HPLC/GFAA method described in Section 4 and included in Appendix 3. Following a detailed review of this method by Amoco, it was determined that although the method is capable of calculating concentration results for each species, these concentration results do not meet accepted USEPA criteria for evaluating accuracy, precision, and bias. However, the presence of arsenic species can also be demonstrated using peak height responses observed on the GFAA. The use of peak heights allows the speciation results to be reported in terms of an analytical unit that can be verified using standard QA/QC methodologies.

A peak height is the detection recorded at a specific retention time in response to the presence of an analyte by the GFAA and is expressed in microvolts (μV). An example chromatogram generated using the HPLC/GFAA is presented as Figure 5-1. The cumulative significant peak heights are normalized based on the response factor of a standard arsenic(III) or arsenic(V) analyzed using the HPLC/GFAA. Thus, the normalized peak height is the GFAA detector response to a peak at a retention time, adjusted for a standard expected response, for the arsenic species of interest.

QA/QC RESULTS

Two different types of QA/QC samples were collected during the arsenic speciation study. First, internal QA/QC analyses were performed by the two laboratories taking part in

the study. Secondly, Amoco provided field samples for analysis to serve as an external QA/QC check. The field QA/QC samples consisted of:

- duplicate samples;
- spike additions of arsenic(III) and arsenic(V) to wastewater samples (spike addition); and,
- deionized water blank samples.

The results of these samples are discussed below.

Amoco Naperville Research and Development Laboratory Results

Amoco analyzed samples for total arsenic using USEPA Method 206.2 To verify the QA/QC data for these samples they were validated as shown in Appendix 5. The accuracy and precision of the method used was acceptable based performance data provided by the laboratory on the percent recoveries for spiked samples and relative percent difference (RPD) between identical samples. These data agreed with the estimated arsenic detection limit for this method (1.0 µg/L) and USEPA control limits of 75 to 125 percent recoveries and RPD values of less than 20 percent.

EPL Bio-Analytical Laboratory Results

A complete analytical report for seven weeks of sampling is included in Appendix 4. Detailed descriptions of the analytical and QA/QC methods used are also provided in Appendix 4.

To verify the ability of the method (sample preservation, preparation/extraction, and analyses) to speciate and quantify the arsenic in the Outfall 001 effluent samples, two types

The analytical results presented in Table 5-6 are based on cumulative and normalized peak heights. The cumulative peak heights at the retention times for arsenic(III) in the effluent samples were indistinguishable from the detector baseline electrical signal (i.e., background). Hence, the results for arsenic(III) are presented as less than (<) 20,000 μV. Arsenic(V) cumulative peak height responses were distinguishable from background with responses of 61,000 to 428,000 μV.

The normalized peak heights, given in Table 5-6, confirm that there is no response, based on the arsenic(III) standard response factor, produced at the retention time for arsenic(III). The first group of samples, as evidence by the field blank results, had a normalized peak height detection threshold of <27 μV for arsenic(III) and <94 μV for arsenic(V). The second group of samples had a normalized peak height detection threshold of <212 μV for arsenic(III) and <65 μV for arsenic(V). Arsenic(V) in the samples is producing a response, as evidenced by the normalized peak heights, of 83 to 749 μV. Thus, arsenic(V) is the species in the effluent samples producing a response using the HPLC/GFAA.

EPL has developed, based on their experience and expertise, a procedure to estimate concentrations of arsenic(III) and arsenic(V) as outlined in Appendix 3. Their estimated concentrations of these species in the Amoco effluent samples is given in Table 5-7. Based on these concentration results, arsenic(III) was not detected in the effluent samples.

that the use of cumulative peak heights and normalized peak heights in determining the presence of an arsenic species is appropriate.

SAMPLE RESULTS

The total arsenic analytical results for the seven samples are provided in Table 5-5. The average total arsenic reported by Laboratory 1 was 16.2 μg/L. The average total arsenic reported by Laboratory 2 was 17.1 μg/L. The average of the difference between laboratories for the seven sampling events was 9 percent. These results indicate an acceptable level of reproducability of total arsenic analyses between laboratories. The results for total arsenic detected in the treated effluent are less than the federal primary drinking water standard for total arsenic of 50 μg/L.

The results of the speciation analysis of the treated effluent are presented based on three types of data for the samples:

- Cumulative peak heights which represents the sum of the detector response measured as an electrical signal at the retention times for the arsenic species;
- Normalized peak heights which is the cumulative peak height for the individual arsenic species adjusted for the response factor for the proper arsenic standard; and
- EPL's estimate of concentrations for each arsenic species in the effluent samples.

The confidence in the effluent sample results, as expressed by the detector response, based on the QA/QC evaluation, is high.

a species is not present, i.e. a result of 100 percent is not possible in reality. These results are within EPL's normal QA/QC limits and within USEPA suggested recoveries for analytical methods.

The results of the three spiked field samples submitted by Amoco over the seven week sampling period are presented in Table 5-2. The average ratio for these spiked samples is well within the acceptable result based on the method for preparing the field spike and analytical variability. These fractions indicate the method's ability to speciate and analytically detect the relative proportions of arsenic(III) and arsenic(V) and that the Outfall 001 effluent matrix was not adversely impacting this analysis.

To evaluate the reproducability of the analyses, Amoco submitted duplicate field samples on three of the sampling events. The relative percent difference (RPD) value was calculated for each sample pair based on concentration for total arsenic and normalized peak heights for arsenic(III) and arsenic(V). The RPD values, reported in Table 5-3, indicated average RPD values of 9, 0, and 32 percent for total arsenic, arsenic(III), and arsenic(V), respectively. These values for RPDs are within acceptable levels.

The final type of QA/QC samples tested were field blanks of deionized water passed through the sample equipment. Arsenic (total or either species) was not detected in any of the three blank samples, as shown in Table 5-4. These results indicate that the sampling equipment, sample handling, and sample digestion and preparation did not bias the reported analytical results.

Therefore, it is concluded that the EPL HPLC/GFAA arsenic speciation method is capable of extracting, speciating, and detecting arsenic(III) and arsenic(V). It is also concluded

of internal QA/QC samples were prepared. The laboratory prepared internal matrix spike samples and Amoco submitted as "blind" samples, external matrix spike samples. Internal matrix spike samples were prepared by adding known quantities of aqueous reference standard for arsenic(III) or arsenic(V) to an accurate volume of a sample before the preparation/extraction steps. External spike samples were prepared by Amoco by adding roughly equal amounts (approximately 500 μg/L each) of arsenic(III) and arsenic(V) to the effluent sample already contained in the sample bottle.

A summary of these results for the internal matrix spike samples is provided in Table 5-1. The theoretical concentration in Table 5-1 is the known amount of the arsenic(III) or arsenic(V) aqueous reference standard that was added to the sample. Table 5-1 also presents normalized peak height results for arsenic(III) and arsenic(V). These peak heights are used to calculate the relative amounts of a species in the sample. This is achieved by first correcting the normalized peak height for each species to account the cumulative peak height that was recorded for the presence of arsenic species observed in the sample prior to the addition of the spike of arsenic. Thus, if the method is performing as desired, one would expect internal QA/QC spiked samples to return a relative amount of close to one hundred percent for whichever species was used in the spike. The results in Table 5-1 demonstrate the presence of the arsenic species at the expected equivalent response for the species. The relative amount of arsenic(III) ranged from 92.2 to 98.6 percent compared to a theoretical amount of 100 percent. The relative amount of arsenic(V) ranged from 83.5 to 91.0 percent compared to a theoretical amount of 100 percent. It should be noted that due to the quantitation limits of the instruments, there will always be a minimum peak height even when

TABLE D5-1. SUMMARY OF ARSENIC SPECIATION STUDY QA/QC INTERNAL SPIKE SAMPLE ANALYTICAL RESULTS

SAMPLE DATE	SAMPLE NAME	ARSENIC FORM	THEORETICAL CONCENTRATION (a) (µg/L)	NORMALIZED PEAK HEIGHT (b) (µV)	RELATIVE AMOUNT (c) (%)
26-Apr-94	1-B-O	Arsenic (III)	50	569	> 95.5
17-May-94	3-B-O	Arsenic (III)	54	2,102	> 98.6
01-Jun-94	6-B-O	Arsenic (III)	54	1,154	> 92.2
26-Apr-94	1-B-O	Arsenic (V)	55	952	> 91.0
17-May-94	3-B-O	Arsenic (V)	54	1,529	> 87.8
01-Jun-94	6-B-O	Arsenic (V)	54	1,074	> 83.5

NOTES:
(a) Amount added to sample. The initial sample concentrations have been subtracted from the results presented here.
(b) Normalized peak height = (HPLC/GFAA sample peak height)/(species standard response factor)
(c) Relative amount = (Normalized peak height As for one species)/(Normalized peak height for both species)
(f) Average values for relative amounts are based on absolute value of percents listed.

TABLE D5-2. SUMMARY OF ARSENIC SPECIATION STUDY QA / QC FIELD SPIKE SAMPLE ANALYTICAL RESULTS

SAMPLE DATE	SAMPLE DESCRIPTION	ANALYTICAL RESULTS (a)							
		LABORATORY 1		LABORATORY 2					
						ARSENIC(III)		ARSENIC(V)	
		SAMPLE NAME	TOTAL ARSENIC CONCENTRATION (µg/L)	SAMPLE NAME	TOTAL ARSENIC CONCENTRATION (µg/L)	NORMALIZED PEAK HEIGHT (µV)	FRACTION OF TOTAL (c)	NORMALIZED PEAK HEIGHT (µV)	FRACTION OF TOTAL (c)
04-May-94	Outfall 001; spike addition (b)	2-N-2	420	2-B-2	510	686	59%	476	41%
26-May-94	Outfall 001; spike addition (b)	5-N-2	250	5-B-2	620	2,037	62%	1,251	38%
07-Jun-94	Outfall 001; spike addition (b)	7-N-2	430	7-B-2	450	1,219	63%	731	37%
AVERAGE			367		527		61%		39%

NOTES:

(a) Laboratory 1 - Amoco Naperville R & D, Naperville, Illinois
Laboratory 2 - EPL Bio-Analytical, Inc., Harrisburg, Illinois
(b) Spiked with approximately 500 ug/L arsenic equally split between arsenic (III) and arsenic (V).
(c) Fraction of Total based on Relative Amounts of each species calculated below:
Relative Amount = ((Normalized peak height for one species)/(normalized peak height for both species)) x 100
(d) Based on the process of spike addition and inherent analytical variability, a ratio result of 61% to 39% is acceptable.

(µg/L) = micrograms/Liter (concentration)
(µV) = microvolts (GFAA detector response to peak at retention time for arsenic species)

TABLE D5-3. SUMMARY OF ARSENIC SPECIATION STUDY DUPLICATE SAMPLE ANALYTICAL RESULTS

SAMPLE DATE	SAMPLE DESCRIPTION	ANALYTICAL RESULTS (a)									
		LABORATORY 1			LABORATORY 2						
		SAMPLE NAME	ARSENIC TOTAL CONC. (µg/L)	RPD (b) (%)	SAMPLE NAME	ARSENIC TOTAL CONC. (µg/L)	RPD (b) (%)	NORMALIZED PEAK HEIGHT ARSENIC (III) (c) (µV)	RPD (b) (%)	NORMALIZED PEAK HEIGHT ARSENIC (V) (d) (µV)	RPD (b) (%)
04-May-94	Outfall 001; 24-hour composite	2-N-0	15.6	0%	2-B-0	19	5%	<27	0%	155	26%
	Outfall 001; duplicate	2-N-1	15.6		2-B-1	20		<27		119	
26-May-94	Outfall 001; 24-hour composite	5-N-0	16	6%	5-B-0	11	0%	<212	0%	292	21%
	Outfall 001; duplicate	5-N-1	15		5-B-1	11		<212		237	
07-Jun-94	Outfall 001; 24-hour composite	7-N-0	13	8%	7-B-0	16	21%	<212	0%	286	48%
	Outfall 001; duplicate	7-N-1	12		7-B-1	13		<212		176	
AVERAGE				5%			9%		0%		32%

NOTES:

(a) Laboratory 1 – Amoco Naperville R & D, Naperville, Illinois
Laboratory 2 – EPL Bio-Analytical, Inc., Harrisburg Illinois

(b) RPD – Relative Percent Difference calculated as follows:
RPD = (|r-d|/((r+d)/2))*100
where :
r = result, ug/L
d = duplicate, ug/L

(c) Arsenic (III) threshold normalized peak height:
Group 1 (1-B & 2-B) samples is <27 µV
Group 2 (3-B through 7-B) samples is <212 µV

(d) Arsenic (V) threshold normalized peak height:
Group 1 (1-B & 2-B) samples is <94 µV
Group 2 (3-B through 7-B) samples is <65 µV

(µg/L) = micrograms/Liter (concentration)

(µV) = microvolts (GFAA detector response to peak at retention time for arsenic species)

TABLE D5-4. SUMMARY OF ARSENIC SPECIATION STUDY QA / QC BLANK SAMPLE ANALYTICAL RESULTS

SAMPLE DATE	SAMPLE DESCRIPTION	ANALYTICAL RESULTS (a)					
		LABORATORY 1		LABORATORY 2			
		SAMPLE NAME	TOTAL ARSENIC CONCENTRATION (μg/L)	SAMPLE NAME	TOTAL ARSENIC CONCENTRATION (μg/L)	NORMALIZED PEAK HEIGHT ARSENIC (III) (μV)	NORMALIZED PEAK HEIGHT ARSENIC (V) (μV)
04-May-94	Deionized water blank	2-N-3	< 1	2-B-3	< 5	< 27	< 94
26-May-94	Deionized water blank	5-N-3	4	5-B-3	< 5	< 212	< 94
07-Jun-94	Deionized water blank	7-N-3	< 1	7-B-3	< 5	< 212	< 35

NOTES:

(a) Laboratory 1 - Amoco Naperville R & D, Naperville, Illinois
Laboratory 2 - EPL Bio-Analytical, Inc., Harrisburg, Illinois

(μg/L) = micrograms/Liter (concentration)
(μV) = microvolts (GFAA detector response to peak at retention time for arsenic species)

TABLE D5-5. SUMMARY OF TOTAL ARSENIC ANALYTICAL RESULTS

SAMPLE DATE	SAMPLE DESCRIPTION	ANALYTICAL RESULTS (a)				DIFFERENCE BETWEEN LABORATORY 1 & 2 FOR ARSENIC, TOTAL
		LABORATORY 1		LABORATORY 2		
		SAMPLE NAME	TOTAL ARSENIC CONCENTRATION (µg/L)	SAMPLE NAME	TOTAL ARSENIC CONCENTRATION (µg/L)	
26-Apr-94	Outfall 001; 24-hour composite	1-N-0	13	1-B-0	20	54%
04-May-94	Outfall 001; 24-hour composite	2-N-0	15.6	2-B-0	19	22%
10-May-94	Outfall 001; 24-hour composite	3-N-0	18	3-B-0	18	0%
17-May-94	Outfall 001; 24-hour composite	4-N-0	17	4-B-0	19	12%
26-May-94	Outfall 001; 24-hour composite	5-N-0	16	5-B-0	11	-31%
01-Jun-94	Outfall 001; 24-hour composite	6-N-0	21	6-B-0	17	-19%
07-Jun-94	Outfall 001; 24-hour composite	7-N-0	13	7-B-0	16	23%
AVERAGE			16.2		17.1	9%

NOTES:

(a) Laboratory 1 - Amoco Naperville R & D, Naperville, Illinois based on USEPA Method 206.2
Laboratory 2 - EPL Bio-Analytical, Inc., Harrisburg, Illinois based on USEPA Method 206.2

(µg/L) = micrograms/Liter (concentration)

TABLE D5-6. SUMMARY OF ARSENIC SPECIATION STUDY SAMPLE ANALYTICAL RESULTS

SAMPLE DATE	SAMPLE DESCRIPTION	LABORATORY 2 (a)				
		SAMPLE NAME	ARSENIC (III)		ARSENIC (V)	
			CUMULATIVE PEAK HEIGHT (b) (μV)	NORMALIZED PEAK HEIGHT (c) (μV)	CUMULATIVE PEAK HEIGHT (b) (μV)	NORMALIZED PEAK HEIGHT (c) (μV)
26-Apr-94	Outfall 001; 24-hour composite	1-B-0	< 20,000	< 27	61,549	83
04-May-94	Outfall 001; 24-hour composite	2-B-0	< 20,000	< 27	114,388	155
10-May-94	Outfall 001; 24-hour composite	3-B-0	< 20,000	< 212	427,751	749
17-May-94	Outfall 001; 24-hour composite	4-B-0	< 20,000	< 212	215,788	378
26-May-94	Outfall 001; 24-hour composite	5-B-0	< 20,000	< 212	166,545	292
01-Jun-94	Outfall 001; 24-hour composite	6-B-0	< 20,000	< 212	180,449	316
07-Jun-94	Outfall 001; 24-hour composite	7-B-0	< 20,000	< 212	163,151	286

NOTES:

(a) Laboratory 2 – EPL Bio-Analytical, Inc., Harrisburg, Illinois using HPLC/GFAA method
(b) Cumulative peak height = sum of individual peaks
The result of <20,000 μV indicated that the response was indistinguishable from background
(c) Normalized peak height = (HPLC/GFAA Sample peak height) ÷ (Species standard response factor)
The normalized peak height threshold is:
Group 1 Samples Arsenic (III) = <27 μV
Group 2 Samples Arsenic (III) = <212 μV
Group 1 Samples Arsenic (V) = <94 μV
Group 2 Samples Arsenic (V) = <65 μV

(μV) = microvolts (GFAA detector response to peak at retention time for arsenic species)

TABLE D5-7. SUMMARY OF ARSENIC SPECIATION STUDY SAMPLE ANALYTICAL RESULTS AS CONCENTRATIONS

SAMPLE DATE	SAMPLE DESCRIPTION	LABORATORY 2 (a)		
		SAMPLE NAME	ARSENIC (III) (b) (μg/L)	ARSENIC (V) (μg/L)
26-Apr-94	Outfall 001; 24-hour composite	1-B-0	< 5	20
04-May-94	Outfall 001; 24-hour composite	2-B-0	< 5	19
10-May-94	Outfall 001; 24-hour composite	3-B-0	< 5	18
17-May-94	Outfall 001; 24-hour composite	4-B-0	< 5	19
26-May-94	Outfall 001; 24-hour composite	5-B-0	< 5	11
01-Jun-94	Outfall 001; 24-hour composite	6-B-0	< 5	16
07-Jun-94	Outfall 001; 24-hour composite	7-B-0	< 5	16

NOTES:

(a) Laboratory 2 - EPL Bio-Analytical, Inc., Harrisburg, Illinois using HPLC/GFAA method and their procedures for estimating concentrations of arsenic species.

(b) The HPLC/GFAA detection limit for arsenic, based on EPL's experience, is 5 μg/L.

(μg/L) = micrograms per Liter

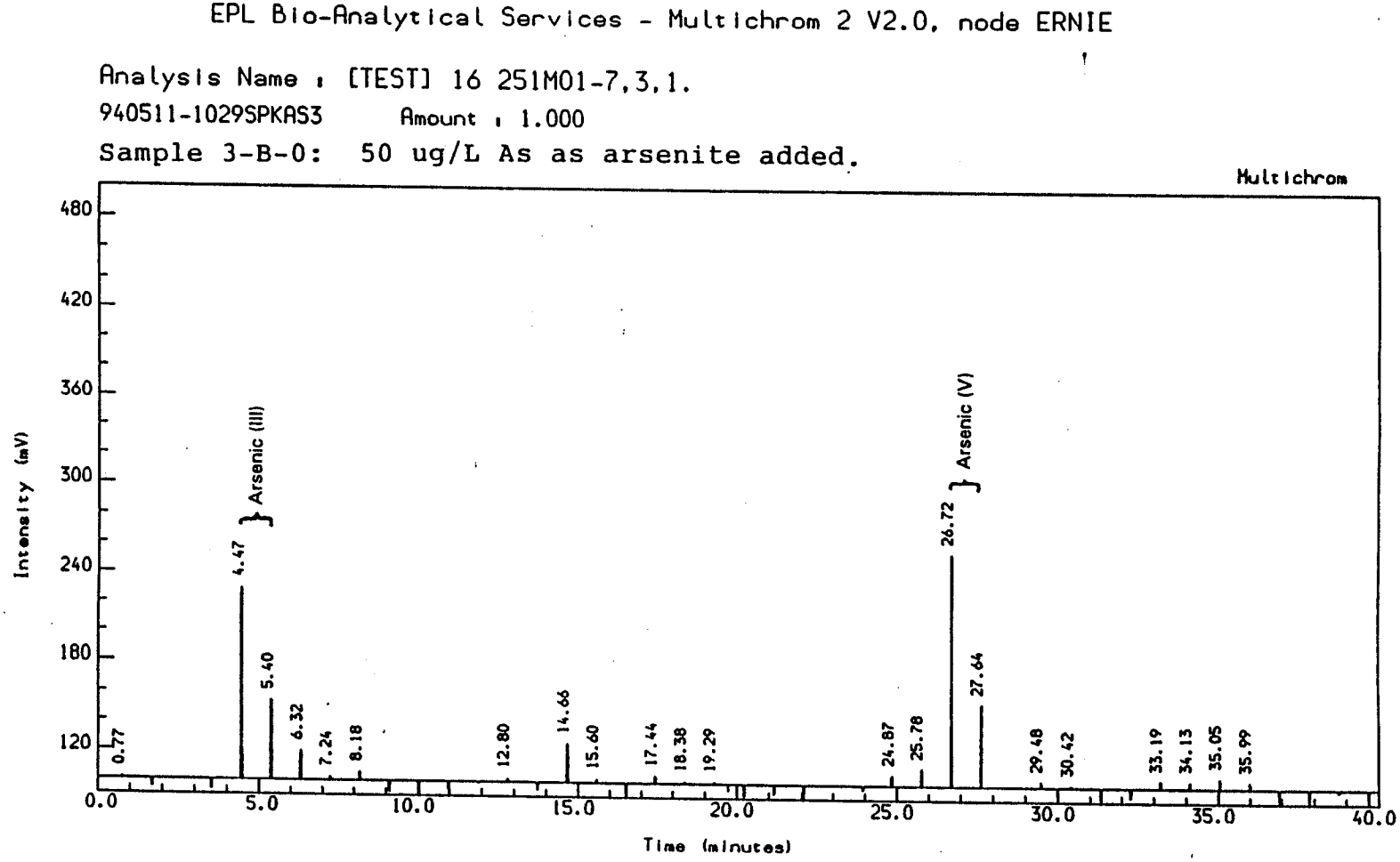


FIGURE 5-1. EXAMPLE CHROMATOGRAM FOR INTERNAL SPIKE OF ARSENIC (III) EFFLUENT SAMPLE 3-B-0

SECTION 6

CONCLUSIONS

The Indiana Water Quality Standards (IWQS) have established aquatic life and human health criteria for arsenic(III). Amoco has determined that these criteria are not applicable to its treated effluent because arsenic(III) is not present in the effluent. This conclusion is based on the findings presented in this report. The findings are summarized below:

- Integrating arsenic chemistry with knowledge of the biological (aerobic) wastewater treatment system, augmented by pH and Eh results, arsenic(V) is the only species of arsenic present in Amoco's treated effluent.

- The reproducability between laboratories in quantifying total arsenic in the effluent samples, using USEPA Method 206.2, was very good, hence we have a high degree of confidence in the laboratories to perform analyses.

- The QA/QC results from the HPLC/GFAA arsenic speciation method indicate satisfactory performance for the effluent samples in extraction, speciation, and detection of arsenic. This is based on the results of:
  - response factors for arsenic species standards;
  - acceptable relative amounts recovered of arsenic species from internal spike samples;
  - adequate relative percent differences for duplicate samples; and
  - no responses for either arsenic species for blank samples.

- The results for the Amoco field spike samples confirm that the HPLC/GFAA arsenic speciation method is applicable to Outfall 001 effluent matrix.

- Arsenic(III) speciation results indicate that this form of arsenic is not present in the effluent samples based on normalized peak heights at the retention time for arsenic(III). The cumulative peak heights at this retention time for arsenic(III) were indistinguishable from the detector baseline (background).

- Arsenic(V) speciation results indicate that this form is present in the effluent. The response of the GFAA at the retention time for arsenic(V) is easily distinguishable from detector baseline (background).

Based on arsenic chemistry, the source of refinery water, experience with the wastewater treatment system, augmented by analytical results for pH and Eh, the only species of arsenic present in Amoco's treated effluent is arsenic(V). The analytical results using the HPLC/GFAA arsenic speciation method demonstrate that arsenic(III) is not detected and that arsenic(V) is detected. These two findings together confirm that the IWQS for arsenic(III) does

not apply to the Amoco Whiting Refinery Outfall 001 effluent. Therefore, Amoco's renewed permit should not contain a limit for arsenic.

APPENDIX 1

STANDARD METHODS FOR THE EXAMINATION OF WATER AND WASTEWATER, 1992, 18th Edition, APHA, AWWA, WEF

ARSENIC ANALYTICAL METHODS

9. Bibliography

RENSHAW, G.D. 1973. The determination of barium by flameless atomic absorption spectrophotometry using a modified graphite tube atomizer. *Atomic Absorption Newsletter* 12:158.

YANAGISAWA, M., T. TAKEUCHI & M. SUZUKI. 1973. Flameless atomic absorption spectrometry of antimony. *Anal. Chim. Acta* 64:381.

RATTONETTI, A. 1974. Determination of soluble cadmium, lead, silver and indium in rainwater and stream water with the use of flameless atomic absorption. *Anal. Chem.* 46:739.

HENN, E.L. 1975. Determination of selenium in water and industrial effluents by flameless atomic absorption. *Anal. Chem.* 47:428.

MARTIN, T.D. & J.F. KOPP. 1975. Determining selenium in water, wastewater, sediment and sludge by flameless atomic absorption spectrometry. *Atomic Absorption Newsletter* 14:109.

MARUTA, T., K. MINEGISHI & G. SUDOH. 1976. The flameless atomic absorption spectrometric determination of aluminum with a carbon atomization system. *Anal. Chim. Acta* 81:313.

CRANSTON, R.E. & J.W. MURRAY. 1978. The determination of chromium species in natural waters. *Anal. Chim. Acta* 99:275.

HOFFMEISTER, W. 1978. Determination of iron in ultrapure water by atomic absorption spectroscopy. *Z. Anal. Chem.* 50:289.

LAGAS, P. 1978. Determination of beryllium, barium, vanadium and some other elements in water by atomic absorption spectrometry with electrothermal atomization. *Anal. Chim. Acta* 98:261.

CARRONDO, M.J.T., J.N. LESTER & R. PERRY. 1979. Electrothermal atomic absorption determination of total aluminum in waters and waste waters. *Anal. Chim. Acta* 111:291.

NAKAHARA, T. & C.L. CHAKRABARTI. 1979. Direct determination of traces of molybdenum in synthetic sea water by atomic absorption spectrometry with electrothermal atomization and selective volatilization of the salt matrix. *Anal. Chim. Acta* 104:99.

TIMINAGA, M. & Y. UMEZAKI. 1979. Determination of submicrogram amounts of tin by atomic absorption spectrometry with electrothermal atomization. *Anal. Chim. Acta* 110:55.

3114 METALS BY HYDRIDE GENERATION/ATOMIC ABSORPTION SPECTROMETRY*

3114 A. Introduction

For general introductory material on atomic absorption spectrometric methods, see Section 3111A.

Two methods are presented in this section: A manual method and a continuous-flow method especially recommended for selenium. Continuous-flow automated systems are preferable to manual hydride generators because the effect of sudden hydrogen generation on light-path transparency is removed and any blank response from contamination of the HCl reagent by the elements being determined is incorporated into the background base line.

* Approved by Standard Methods Committee, 1989.

3114 B. Manual Hydride Generation/Atomic Absorption Spectrometric Method

1. General Discussion

a. Principle: This method is applicable to the determination of arsenic and selenium by conversion to their hydrides by sodium borohydride reagent and aspiration into an atomic absorption atomizer.

Arsenous acid and selenous acid, the As(III) and Se(IV) oxidation states of arsenic and selenium, respectively, are instantaneously converted by sodium borohydride reagent in acid solution to their volatile hydrides. The hydrides are purged continuously by argon or nitrogen into an appropriate atomizer of an atomic absorption spectrometer and converted to the gas-phase atoms. The sodium borohydride reducing agent, by rapid generation of the elemental hydrides in an appropriate reaction cell, minimizes dilution of the hydrides by the carrier gas and provides rapid, sensitive determinations of arsenic and selenium.

CAUTION: *Arsenic and selenium and their hydrides are toxic. Handle with care.*

At room temperature and solution pH values of 1 or less, arsenic acid, the As(V) oxidation state of arsenic, is reduced relatively slowly by sodium borohydride to As(III), which is then instantaneously converted to arsine. The arsine atomic absorption peaks commonly are decreased by one-fourth to one-third for As(V) when compared to As(III). Determination of total arsenic requires that all inorganic arsenic compounds be in the As(III) state. Organic and inorganic forms of arsenic are first oxidized to As(V) by acid digestion. The As(V) then is quantitatively reduced to As(III) with sodium or potassium iodide before reaction with sodium borohydride.

Selenic acid, the Se(VI) oxidation state of selenium, is not measurably reduced by sodium borohydride. To determine total selenium by atomic absorption and sodium borohydride, first reduce Se(VI) formed during the acid digestion procedure to Se(IV), being careful to prevent reoxidation by chlorine. Efficiency of reduction depends on temperature, reduction time, and HCl concentration. For 4*N* HCl, heat 1 h at 100°C. For 6*N* HCl, boiling for 10 min is sufficient.[1-3] Alternatively, autoclave samples in sealed containers at 121°C for 1 h. NOTE: Autoclaving in sealed containers may result in incomplete reduction, apparently due to the buildup of chlorine gas. To obtain equal instrument

responses for reduced Se(VI) and Se (IV) solutions of equal concentrations, manipulate HCl concentration and heating time. For further details, see Section 3500-Se.

b. Equipment selection:

Certain atomic absorption atomizers and hydride reaction cells are available commercially for use with the sodium borohydride reagent. A functional system is presented in Figure 3114:1. Irrespective of the hydride reaction cell-atomizer system selected, it must meet the following quality-control considerations: (*a*) it must provide a precise and reproducible standard curve between 0 and 20 µg As or Se/L and an instrumental detection limit between 0.1 and 0.5 µg As or Se/L; (*b*) when carried through the entire procedure, oxidation state couples [As (III) - As (V) or Se (IV) - Se (VI)] must cause equal instrument response; and (*c*) sample digestion must yield 80% or greater recovery of added cacodylic acid (dimethyl arsinic acid) and 90% or greater recovery of added As(III), As(V), Se(VI), or Se(IV).

Three types of atomic absorption atomizers commonly are used in the measurement of arsenic and selenium. Most instrument manufacturers can provide a Boling-type burner for argon (or nitrogen)-air entrained-hydrogen flames. Alternatively use an externally heated quartz cell or a quartz cell with an internal fuel rich oxygen-hydrogen or air-hydrogen flame. Quartz atomization cells provide for the most sensitive arsenic and selenium hydride determinations and minimize background noise associated with the argon-air entrained-hydrogen flame.

c. Digestion techniques: Waters and wastewaters may contain varying amounts of organic arsenic compounds and inorganic compounds of As(III), As(V), Se(IV), and Se(VI). To measure total arsenic and selenium in these samples requires sample digestion to solubilize particulate forms and oxidize reduced forms of arsenic and selenium and to convert any organic compounds to inorganic ones. Organic selenium compounds rarely have been demonstrated in water. It is left to the experienced analyst's judgment whether sample digestion is required.

Two digestion procedures are provided in ¶ 4*c* below. Consider sulfuric-nitric-perchloric acid digestion or sulfuric-nitric acid digestion as providing a measure of total recoverable arsenic rather than total arsenic because they do not completely convert certain organic arsenic compounds to As(V). The sulfuric-nitric-perchloric acid digestion effectively destroys organics and most particulates in untreated wastewaters or solid samples. The potassium persulfate digestion (¶ 4*d*) is effective for converting organic arsenic and selenium compounds to As(V) and Se(VI) in potable and surface waters and in most wastewaters.[4]

The HCl-autoclave reduction of Se(VI) described above is an effective digestion procedure for total inorganic Se; however, it has not been found effective for converting benzene substituted selenium compounds to inorganic selenium.

d. Interferences: Interferences are minimized because the As and Se hydrides are removed from the solution containing most potential interfering substances. Slight response variations occur when acid matrices are varied. Control these variations by treating standards and samples in the same manner. Low concentrations of noble metals (approximately 100 µg/L of Ag, Au, Pt, Pd, etc.), concentrations of copper, lead, and nickel at or greater than 1 mg/L, and concentrations between 0.1 and 1 mg/L of hydride-forming elements (Bi, Sb, Sn, and Te) may suppress the response of As and Se hydrides. Interference by transition metals depends strongly on HCl concentration. Interferences are less pronounced at 4 to 6*N* HCl than at lower concentrations.[5] The

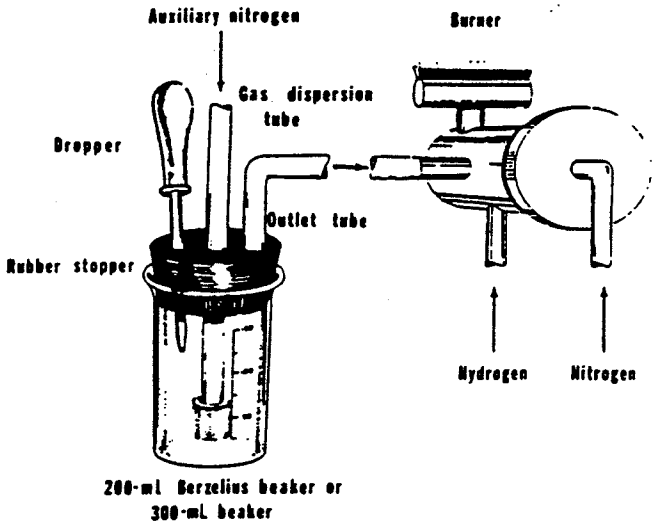


Figure 3114:1. Manual reaction cell for producing As and Se hydrides.

presence of As or Se in each other's matrices can cause similar suppression. Reduced nitrogen oxides resulting from HNO_3 - digestion and nitrite also can suppress instrumental response for both elements. Large concentrations of iodide interfere with the Se determination by reducing Se to its elemental form. Do not use any glassware for determining Se that has been used for iodide reduction of As(V).

To prevent chlorine gas produced in the reduction of Se(VI) to Se(IV) from reoxidizing the Se(IV), generate the hydride within a few hours of the reduction steps or purge the chlorine from the samples by sparging.[6]

Interferences depend on system design and defy quantitative description because of their synergistic effects. Certain waters and wastewaters can contain interferences in sufficient concentration to suppress absorption responses of As and Se. For representative samples in a given laboratory and for initial analyses of unknown wastewaters, add appropriate inorganic forms of As or Se to digested sample portions and measure recovery. If average recoveries are less than 90%, consider using alternative analytical procedures.

e. Detection limit and optimum concentration range: For both arsenic and selenium, analyzed by aspiration into a nitrogen-hydrogen flame after reduction, the method detection limit is 0.002 mg/L and the optimum concentration range 0.002 to 0.02 mg/L.

2. Apparatus

a. Atomic absorption spectrometer equipped with gas flow meters for argon (or nitrogen) and hydrogen, As and Se electrodeless discharge lamps with power supply, background correction at measurement wavelengths, and appropriate strip-chart recorder. A good-quality 10-mV recorder with high sensitivity and a fast response time is needed.

b. Atomizer: Use one of the following:

1) *Boling-type burner head* for argon (or nitrogen)-air entrained-hydrogen flame.

2) *Cylindrical quartz cell*, 10 to 20 cm long, electrically heated by external nichrome wire to 800 to 900°C.[7]

3) *Cylindrical quartz cell* with internal fuel rich hydrogen-oxygen (air) flame.*

The sensitivity of quartz cells deteriorates over several months of use. Sensitivity sometimes may be restored by treatment with 40% HF. CAUTION: *HF is extremely corrosive. Avoid all contact with exposed skin. Handle with care.*

c. Reaction cell for producing As or Se hydrides: See Figure 3114:1. A commercially available system is acceptable if it utilizes liquid sodium borohydride reagents; accepts samples digested in accordance with ¶s 4*c*, *d*, and *e*; accepts 4 to 6*N* HCl; and is efficiently and precisely stirred by the purging gas and/or a magnetic stirrer.

d. Eye dropper or syringe capable of delivering 0.5 to 3.0 mL sodium borohydride reagent. Exact and reproducible addition is required so that production of hydrogen gas does not vary significantly between determinations.

e. Vent: See Section 3111A.6*f*.

3. Reagents

a. Sodium borohydride reagent: Dissolve 8 g $NaBH_4$ in 200 mL 0.1*N* NaOH. Prepare fresh daily.

b. Sodium iodide prereductant solution: Dissolve 50 g NaI in 500 mL water. Prepare fresh daily. Alternatively use an equivalent KI solution.

c. Sulfuric acid, 18*N*.

d. Sulfuric acid, 2.5*N*: Cautiously add 35 mL conc H_2SO_4 to about 400 mL water, let cool, and adjust volume to 500 mL.

e. Potassium persulfate, 5% solution: Dissolve 25 g $K_2S_2O_8$ in water and dilute to 500 mL. Store in glass and refrigerate. Prepare weekly.

f. Nitric acid, HNO_3, conc.

g. Perchloric acid, $HClO_4$, conc.

h. Hydrochloric acid, HCl, conc.

i. Argon (or nitrogen), commercial grade.

j. Hydrogen, commercial grade.

k. Arsenic(III) solutions:

1) *Stock As(III) solution:* Dissolve 1.320 g arsenic trioxide, As_2O_3, in water containing 4 g NaOH. Dilute to 1 L: 1.00 mL = 1.00 mg As(III).

2) *Intermediate As(III) solution:* Dilute 10 mL stock As solution to 1000 mL with water containing 5 mL conc HCl: 1.00 mL = 10.0 µg As(III).

3) *Standard As(III) solution:* Dilute 10 mL intermediate As(III) solution to 1000 mL with water containing the same concentration of acid used for sample preservation (2 to 5 mL conc HNO_3): 1.00 mL = 0.100 µg As(III). Prepare diluted solutions daily.

l. Arsenic(V) solutions:

1) *Stock As(V) solution:* Dissolve 1.534 g arsenic pentoxide, As_2O_5, in distilled water containing 4 g NaOH. Dilute to 1 L: 1.00 mL = 1.00 mg As(V).

2) *Intermediate As(V) solution:* Prepare as for As(III) above: 1.00 mL = 10.0 µg As(V).

3) *Standard As(V) solution:* Prepare as for As(III) above; 1.00 mL = 0.100 µg As(V).

m. Organic arsenic solutions:

1) *Stock organic arsenic solution:* Dissolve 1.842 g dimethylarsinic acid (cacodylic acid), $(CH_3)_2AsOOH$, in water containing 4 g NaOH. Dilute to 1 L: 1.00 mL = 1.00 mg As. [NOTE: Check purity of cacodylic acid reagent against an intermediate arsenic standard (50 to 100 mg As/L) using flame atomic absorption.]

2) *Intermediate organic arsenic solution:* Prepare as for As(III) above: 1.00 mL = 10.0 µg As.

3) *Standard organic arsenic solution:* Prepare as for As(III) above; 1.00 mL = 0.100 µg As.

n. Selenium(IV) solutions:

1) *Stock Se(IV) solution:* Dissolve 2.190 g sodium selenite, Na_2SeO_3, in water containing 10 mL HCl and dilute to 1 L: 1.00 mL = 1.00 mg Se(IV).

2) *Intermediate Se(IV) solution:* Dilute 10 mL stock Se(IV) to 1000 mL with water containing 10 mL conc HCl: 1.00 mL = 10.0 µg Se(IV).

3) *Standard Se(IV) solution:* Dilute 10 mL intermediate Se(IV) solution to 1000 mL with water containing the same concentration of acid used for sample preservation (2 to 5 mL conc HNO_3). Prepare solution daily when checking the equivalency of instrument response for Se(IV) and Se(VI): 1.00 mL = 0.100 µg Se(IV).

o. Selenium(VI) solutions:

1) *Stock Se(VI) solution*: Dissolve 2.393 g sodium selenate, Na_2SeO_4, in water containing 10 mL conc HNO_3. Dilute to 1 L: 1.00 mL = 1.00 mg Se(VI).

2) *Intermediate Se(VI) solution:* Prepare as for Se(IV) above; 1.00 mL = 10.0 µg Se (VI).

3) *Standard Se(VI) solution:* Prepare as for Se(IV) above; 1.00 mL = 0.100 µg Se(VI).

4. Procedure

a. Apparatus setup: Either see Figure 3114:1 or follow manufacturer's instructions. Connect inlet of reaction cell with auxiliary purging gas controlled by flow meter. If a drying cell between the reaction cell and atomizer is necessary, use only anhydrous $CaCl_2$ but not $CaSO_4$ because it may retain SeH_2. Before using the hydride generation/analysis system, optimize operating parameters. Aspirate dilute aqueous solutions of As and Se directly into the flame to facilitate atomizer alignment. Align quartz atomizers for maximum absorbance. Aspirate a blank until memory effects are removed. Establish purging gas flow, concentration and rate of addition of sodium borohydride reagent, solution volume, and stirring rate for optimum instrument response for the chemical species to be analyzed. If a quartz atomizer is used, optimize cell temperature. If sodium borohydride reagent is added too quickly, rapid evolution of hydrogen will unbalance the system. If the volume of solution being purged is too large, the absorption signal will be decreased. Recommended wavelengths are 193.7 and 196.0 nm for As and Se, respectively.

b. Instrument calibration standards: Transfer 0.00, 1.00, 2.00, 5.00, 10.00, 15.00, and 20.00 mL standard solutions of As(III) or Se(IV) to 100-mL volumetric flasks and bring to volume with water containing the same acid concentration used for sample preservation (commonly 2 to 5 mL conc HNO_3/L). This yields blank and standard solutions of 0, 1, 2, 5, 10, 15, and 20 µg As or Se/L. Prepare fresh daily.

c. Preparation of samples and standards for total recoverable arsenic and selenium: Follow general procedures of Section 3030F: alternatively, add 50 mL sample, As(III), or Se(IV) standard to 200-mL Berzelius beaker. (Alternatively, prepare standards by

adding 100 µg/L standard As or Se solutions directly to the beaker and dilute to 50 mL in this beaker). Add 7 mL 18*N* H_2SO_4 and 5 mL conc HNO_3. Add a small boiling chip or glass beads if necessary. Evaporate to SO_3 fumes. Maintain oxidizing conditions at all times by adding small amounts of HNO_3 to prevent solution from darkening. Maintain an excess of HNO_3 until all organic matter is destroyed. Complete digestion usually is indicated by a light-colored solution. Cool slightly, add 25 mL water and 1 mL conc $HClO_4$ and again evaporate to SO_3 fumes to expel oxides of nitrogen. CAUTION: *See Section 3030H for cautions on use of $HClO_4$.* Monitor effectiveness of digestion procedure used by adding 5 mL of standard organic arsenic solution or 5 mL of a standard selenium solution to a 50-mL sample and measuring recovery, carrying standards through entire procedure. To report total recoverable arsenic as total arsenic, average recoveries of cacodylic acid must exceed 80%. Alternatively, use 100-mL micro-kjeldahl flasks for the digestion of total recoverable arsenic or selenium, thereby improving digestion effectiveness. After final evaporation of SO_3 fumes, dilute to 50 mL for arsenic measurements or to 30 mL for selenium measurements.

d. Preparation of samples and standards for total arsenic and selenium: Add 50 mL sample or standard to a 200-mL Berzelius beaker. Add 1 mL 2.5*N* H_2SO_4 and 5 mL 5% $K_2S_2O_8$. Boil gently on a pre-heated hot plate for approximately 30 to 40 min or until a final volume of 10 mL is reached. Do not let sample go to dryness. Alternatively heat in an autoclave at 121°C for 1 h in capped containers. After manual digestion, dilute to 50 mL for subsequent arsenic measurements and to 30 mL for selenium measurements. Monitor effectiveness of digestion by measuring recovery of As or Se as above. If poor recovery of arsenic added as cacodylic acid is obtained, reanalyze using double the amount of $K_2S_2O_8$.

e. Determination of arsenic with sodium borohydride: To 50 mL digested standard or sample in a 200-mL Berzelius beaker (see Figure 3114:1) add 5 mL conc HCl and mix. Add 5 mL NaI prereductant solution, mix, and wait at least 30 min. (NOTE: The NaI reagent has not been found necessary for certain hydride reaction cell designs if a 20 to 30% loss in instrument sensitivity is not important and variables of solution acid conditions, temperatures, and volumes for production of As(V) and arsine can be controlled strictly. Such control requires an automated delivery system; see Section 3114C.)

Attach one Berzelius beaker at a time to the rubber stopper containing the gas dispersion tube for the purging gas, the sodium borohydride reagent inlet, and the outlet to the atomizer. Turn on strip-chart recorder and wait until the base line is established by the purging gas and all air is expelled from the reaction cell. Add 0.5 mL sodium borohydride reagent. After the instrument absorbance has reached a maximum and returned to the base line, remove beaker, rinse dispersion tube with water, and proceed to the next sample or standard. Periodically compare standard As(III) and As(V) curves for response consistency. Check for presence of chemical interferences that suppress instrument response for arsine by treating a digested sample with 10 µg/L As(III) or As(V) as appropriate. Average recoveries should be not less than 90%.

f. Determination of selenium with sodium borohydride: To 30 mL digested standard or sample, or to 30 mL undigested standard or sample in a 200-mL Berzelius beaker, add 15 mL conc HCl and mix. Heat for a predetermined period at 90 to 100°C. Alternatively autoclave at 121°C in capped containers for 60 min, or heat for a predetermined time in open test tubes using a 90 to 100°C hot water bath or an aluminum block digester. Check effectiveness of the selected heating by demonstrating equal instrument responses for calibration curves prepared either from standard Se(IV) or from Se(VI) solutions. Effective heat exposure for converting Se(VI) to Se(IV), with no loss of Se(IV), ranges between 5 and 60 min when open beakers or test tubes are used. Do not digest standard Se(IV) and Se(VI) solutions used for this check of equivalency. After prereduction of Se(VI) to Se(IV), attach Berzelius beakers, one at a time, to the purge apparatus. For each, turn on the strip-chart recorder and wait until the base line is established. Add 0.50 mL sodium borohydride reagent. After the instrument absorbance has reached a maximum and returned to the base line, remove beaker, rinse dispersion tube with water, and proceed to the next sample or standard. Check for presence of chemical interferences that suppress selenium hydride instrument response by treating a digested sample with 10 µg Se (IV)/L. Average recoveries should be not less than 90%.

5. Calculation

Construct a standard curve by plotting peak heights or areas of standards versus concentration of standards. Measure peak heights or areas of samples and read concentrations from curve. If sample was diluted (or concentrated) before sample digestion, apply an appropriate factor. On instruments so equipped, read concentrations directly after standard calibration.

6. Precision and Bias

Single-laboratory, single-operator data were collected for As(III) and organic arsenic by both manual and automated methods, and for the manual determination of selenium. Recovery values (%) from seven replicates are given below:

	As(III)	Org As	Se(IV)	Se(VI)
Manual with digestion	91.8	87.3	—	—
Manual without digestion	109.4	19.4	100.6	110.8
Automated with digestion	99.8	98.4	—	—
Automated without digestion	92.5	10.4	—	—

7. References

1. VIJAN, P.N. & D. LEUNG. 1980. Reduction of chemical interference and speciation studies in the hydride generation-atomic absorption method for selenium. *Anal. Chim. Acta* 120:141.
2. VOTH-BEACH, L.M. & D.E. SHRADER. 1985. Reduction of interferences in the determination of arsenic and selenium by hydride generation. *Spectroscopy* 1:60.
3. JULSHAMN, K., O. RINGDAL, K.-E. SLINNING & O.R. BRAEKKAN. 1982. Optimization of the determination of selenium in marine samples by atomic absorption spectrometry: Comparison of a flameless graphite furnace atomic absorption system with a hydride generation atomic absorption system. *Spectrochim. Acta.* 37B:473.
4. NYGAARD, D.D. & J.H. LOWRY. 1982. Sample digestion procedures for simultaneous determination of arsenic, antimony, and selenium by inductively coupled argon plasma emission spectrometry with hydride generation. *Anal. Chem.* 54:803.

5. WELZ, B. & M. MELCHER. 1984. Mechanisms of transition metal interferences in hydride generation atomic-absorption spectrometry. Part 1. Influence of cobalt, copper, iron and nickel on selenium determination. *Analyst* 109:569.
6. KRIVAN, V., K. PETRICK, B. WELZ & M. MELCHER. 1985. Radiotracer error-diagnostic investigation of selenium determination by hydride-generation atomic absorption spectrometry involving treatment with hydrogen peroxide and hydrochloric acid. *Anal. Chem.* 57:1703.
7. CHU, R.C., G.P. BARRON & P.A.W. BAUMGARNER. 1972. Arsenic determination at submicrogram levels by arsine evolution and flameless atomic absorption spectrophotometric technique. *Anal. Chem.* 44:1476.
8. SIEMER, D.D., P. KOTEEL & V. JARIWALA. 1976. Optimization of arsine generation in atomic absorption arsenic determinations. *Anal. Chem.* 48:836.

8. Bibliography

FERNANDEZ, F.J. & D.C. MANNING. 1971. The determination of arsenic at submicrogram levels by atomic absorption spectrophotometry. *Atomic Absorption Newsletter* 10:86.

JARREL-ASH CORPORATION. 1971. High Sensitivity Arsenic Determination by Atomic Absorption. Jarrel-Ash Atomic Absorption Applications Laboratory Bull. No. As-3.

MANNING, D.C. 1971. A high sensitivity arsenic-selenium sampling system for atomic absorption spectroscopy. *Atomic Absorption Newsletter* 10:123.

BRAMAN, R.S. & C.C. FOREBACK. 1973. Methylated forms of arsenic in the environment. *Science* 182:1247.

CALDWELL, J.S., R.J. LISHKA & E.F. MCFARREN. 1973. Evaluation of a low-cost arsenic and selenium determination of microgram-per-liter levels. *J. Amer. Water Works Assoc.*, 65:731.

AGGETT, J. & A.C. ASPELL. 1976. The determination of arsenic (III) and total arsenic by atomic-absorption spectroscopy. *Analyst* 101:341.

FIORINO, J.A., J.W. JONES & S.G. CAPAR. 1976. Sequential determination of arsenic, selenium, antimony, and tellurium in foods via rapid hydride evolution and atomic absorption spectrometry. *Anal. Chem.* 48:120.

CUTTER, G.A. 1978. Species determination of selenium in natural waters. *Anal. Chem. Acta* 98:59.

GODDEN, R.G. & D.R. THOMERSON. 1980. Generation of covalent hydrides in atomic absorption spectroscopy. *Analyst* 105:1137.

BROWN, R.M., JR., R.C. FRY, J.L. MOYERS, S.J. NORTHWAY, M.B. DENTON & G.S. WILSON. 1981. Interference by volatile nitrogen oxides and transition-metal catalysis in the preconcentration of arsenic and selenium as hydrides. *Anal. Chem.* 53:1560.

SINEMUS, H.W., M. MELCHER & B. WELZ. 1981. Influence of valence state on the determination of antimony, bismuth, selenium, and tellurium in lake water using the hydride AA technique. *Atomic Spectrosc.* 2:81.

DEDINA, J. 1982. Interference of volatile hydride forming elements in selenium determination by atomic absorption spectrometry with hydride generation. *Anal. Chem.* 54:2097.

3114 C. Continuous Hydride Generation/Atomic Absorption Spectrometric Method (PROPOSED)

1. General Discussion

The continuous hydride generator, introduced recently, offers the advantages of simplicity in operation, excellent reproducibility, low detection limits, and high sample volume throughput for selenium analysis following preparations as described in 3500-Se.B or 3114B.4*c* and *d*.

a. Principle: See Section 3114B.

b. Interferences: Free chlorine in hydrochloric acid is a common but difficult-to-diagnose interference. (The amount of chlorine varies with manufacturer and with each lot from the same manufacturer). Chlorine oxidizes the hydride and can contaminate the hydride generator to prevent recoveries under any conditions. When interference is encountered, or preferably before using each new bottle of HCl, eliminate chlorine from a 2.3-L bottle of conc HCl by bubbling with helium (commercial grade, 100 mL/min) for 3 h.

Excess oxidant (peroxide, persulfate, or permanganate) from the total selenium digestion can oxidize the hydride. Follow procedures in 3500-Se.B.2, 3, or 4 to ensure removal of all oxidizing agents before hydride generation.

Nitrite is a common trace constituent in natural and waste waters, and at levels as low as 10 µg/L nitrite can reduce the recovery of hydrogen selenide from Se(IV) by over 50%. Moreover, during the reduction of Se(VI) to Se(IV) by digestion with HCl (3500-Se.B.5), some nitrate is converted to nitrite, which subsequently interferes. When this interference is suspected, add sulfanilamide after sample acidification (or HCl digestion). The diazotization reaction between nitrite and sulfanilamide completely removes the interferent effect (i.e., the standard addition slope is normal).

2. Apparatus

a. Continuous hydride generator: The basic unit is composed of two parts: a precision peristaltic pump, which is used to meter and mix reagents and sample solutions, and the gas-liquid separator. At the gas-liquid separator a constant flow of argon strips out the hydrogen and metal hydride gases formed in the reaction and carries them to the heated quartz absorption cell (3114B.1*b* and 2*b*), which is supported by a metal bracket mounted on top of the regular air acetylene burner head. The spent liquid flows out of the separator via a constant level side drain to a waste bucket. Schematics and operating parameters are shown in Figure 3114:2.

Check flow rates frequently to ensure a steady flow; an uneven flow in any tubing will cause an erratic signal. Remove tubings from pump rollers when not in use. Typical flow rates are: sample, 7 mL/min; acid, 1 mL/min; borohydride reagent, 1 mL/min. Argon flow usually is pre-fixed, typically at 90 mL/min.

b. Atomic absorption spectrometric equipment: See Section 3111A.6.

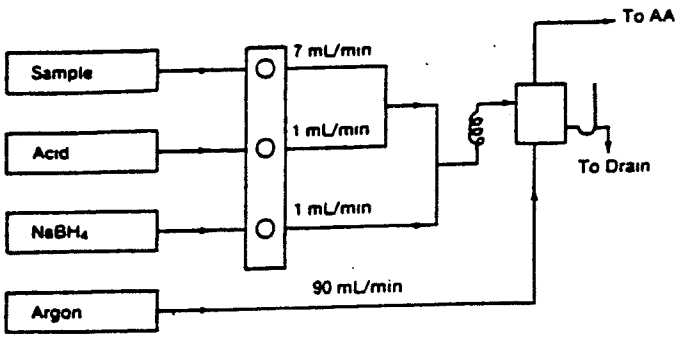


Figure 3114:2. Schematic of a continuous hydride generator.

3. Reagents

a. Hydrochloric acid, HCl, 5 + 1: Handle conc HCl under a fume hood. If necessary, remove free Cl_2 by stripping conc HCl with helium as described above.

b. Borohydride reagent: Dissolve 0.6 g $NaBH_4$ and 0.5 g NaOH in 100 mL water. CAUTION: *Sodium borohydride is toxic, flammable, and corrosive.*

c. Selenium reference standard solution, 1000 mg/L: Use commercially available standard; verify that selenium is Se(IV).

d. Intermediate standard solution, 1 mg/L: Dilute 1 mL reference standard solution to 1 L in a volumetric flask with distilled water.

e. Working standard solutions, 5, 10, 20, 30, and 40 µg/L: Dilute 0.5, 1.0, 2.0, 3.0, and 4.0 mL intermediate standard solution to 100 mL in a volumetric flask.

f. Sulfanilamide solution: Prepare a 2.5% (w/v) solution daily; add several drops conc HCl per 50 mL solution to facilitate dissolution.

4. Procedure

a. Sample preparation: See Section 3500-Se or 3114 B.4*c* and *d* for preparation steps for various Se fractions or total Se.

b. Preconditioning hydride generator: For newly installed tubing, turn on pump for at least 10 to 15 min before instrument calibration. Sample the highest standard for a few minutes to let volatile hydride react with the reactive sites in the transfer lines and on the quartz absorption cell surfaces.

c. Instrument calibration: Depending on total void volume in sample tubing, sampling time of 15 to 20 s generally is sufficient to obtain a steady signal. Between samples, submerge uptake tube in rinse water. Calibrate instrument daily after a 45-min lamp warmup time. Use either the hollow cathode or the electrodeless discharge lamp.

d. Antifoaming agents: Certain samples, particularly wastewater samples containing a high concentration of proteinaceous substances, can cause excessive foaming that could carry the liquid directly into the heated quartz absorption cell and cause splattering of salty deposits onto the windows of the spectrometer. Add a drop of antifoaming agent* to eliminate this problem.

* Dow Corning or equivalent.

e. Nitrite removal: After samples have been acidified, or after acid digestion, add 0.1 mL sulfanilamide solution per 10 mL sample and let react for 2 min.

f. Analysis: Follow manufacturer's instructions for operation of analytical equipment.

5. Calculation

Construct a calibration curve based on absorbance vs. standard concentration. Apply dilution factors on diluted samples.

6. Precision and Bias

Working standards were analyzed together with batches of water samples on a routine production basis. The standards were compounded using chemically pure sodium selenite and sodium selenate. The values of Se(IV) + Se(VI) were determined by converting Se(VI) to Se(IV) by digestion with HCl. Results are tabulated below.

No. Analyses	Mean Se(IV) µg/L	Rel. Dev. %	Se(IV) + Se(VI) µg/L	Rel. Del. %
21	4.3	12	10.3	7
26	8.5	12	19.7	6
22	17.2	7	39.2	8
20	52.8	5	106.0	6

7. Bibliography

REAMER, D.C. & C. VEILOON. 1981. Preparation of biological materials for determination of selenium by hydride generation-AAS. *Anal. Chem.* 53:1192.

SINEMUS, H.W., M. MELCHER & B. WELZ. 1981. Influence of valence state on the determination of antimony, bismuth, selenium, and tellurium in lake water using the hydride AA technique. *Atomic Spectrosc.* 2:81.

RODEN, D.R. & D.E. TALLMAN. 1982. Determination of inorganic selenium species in groundwaters containing organic interferences by ion chromatography and hydride generation/atomic absorption spectrometry. *Anal. Chem.* 54:307.

CUTTER, G. 1983. Elimination of nitrite interference in the determination of selenium by hydride generation. *Anal. Chim. Acta* 149:391.

NARASAKI, H. & M. IKEDA. 1984. Automated determination of arsenic and selenium by atomic absorption spectrometry with hydride generation. *Anal. Chem.* 56:2059.

WELZ, B. & M. MELCHER. 1985. Decomposition of marine biological tissues for determination of arsenic, selenium, and mercury using hydride-generation and cold-vapor atomic absorption spectrometries. *Anal. Chem.* 57:427.

EBDON, L. & S.T. SPARKES. 1987. Determination of arsenic and selenium in environmental samples by hydride generation-direct current plasma-atomic emission spectrometry. *Microchem. J.* 36:198.

EBDON, L. & J.R. WILKINSON. 1987. The determination of arsenic and selenium in coal by continuous flow hydride-generation atomic absorption spectroscopy and atomic fluorescence spectrometry. *Anal. Chim. Acta.* 194:177.

VOTH-BEACH, L.M. & D.E. SHRADER. 1985. Reduction of interferences in the determination of arsenic and selenium by hydride generation. *Spectroscopy* 1:60.

3113 B. Electrothermal Atomic Absorption Spectrometric Method

1. General Discussion

This method is suitable for determination of micro quantities of aluminum, antimony, arsenic, barium, beryllium, cadmium, chromium, cobalt, copper, iron, lead, manganese, molybdenum, nickel, selenium, silver, and tin.

2. Apparatus

a. Atomic absorption spectrometer: See Section 3111A.6*a*. The instrument must have background correction capability.

b. Source lamps: See Section 3111A.6*d*.

c. Graphite furnace: Use an electrically heated device with electronic control circuitry designed to carry a graphite tube or cup through a heating program that provides sufficient thermal energy to atomize the elements of interest. Furnace heat controllers with only three heating steps are adequate only for fresh waters with low dissolved solids content. For salt waters, brines, and other complex matrices, use a furnace controller with up to seven individually programmed heating steps. Fit the furnace into the sample compartment of the spectrometer in place of the conventional burner assembly. Use argon as a purge gas to minimize oxidation of the furnace tube and to prevent the formation of metallic oxides. Use graphite tubes with L'vov platforms to minimize interferences and to improve sensitivity.

d. Readout: See Section 3111A.6*c*.

e. Sample dispensers: Use microliter pipets (5 to 100 µL) or an automatic sampling device designed for the specific instrument.

f. Vent: See Section 3111A.6*f*.

g. Cooling water supply: Cool with tap water flowing at 1 to 4 L/min or use a recirculating cooling device.

h. Membrane filter apparatus: Use an all-glass filtering device and 0.45-µm membrane filters. For trace analysis of aluminum, use device of polypropylene or TFE devices.

3. Reagents

a. Metal-free water: See Section 3111B.3*c*.

b. Hydrochloric acid, HCl, 1 + 1 and conc.

c. Nitric acid, HNO_3, 1 + 1 and conc.

d. Matrix modifiers:

1) *Ammonium nitrate,* 10% (w/v): Dissolve 100 g NH_4NO_3 in water. Dilute to 1000 mL with water.

2) *Ammonium phosphate,* 40%: Dissolve 40 g $(NH_4)_2HPO_4$ in water. Dilute to 100 mL with water.

3) *Calcium nitrate,* 20 000 mg Ca/L: Dissolve 11.8 g $Ca(NO_3)_2 \cdot 4H_2O$ in water. Dilute to 100 mL with water.

4) *Nickel nitrate,* 10 000 mg Ni/L: Dissolve 49.56 g $Ni(NO_3)_2 \cdot 6H_2O$ in water. Dilute to 1000 mL with water.

5) *Phosphoric acid,* 10% (v/v): Dilute 10 mL conc H_3PO_4 to 100 mL with water.

For preparation of other matrix modifiers, see references or follow manufacturers' instructions.

e. Stock metal solutions: Refer to Sections 3111B and 3114.

f. Chelating resin: 100 to 200 mesh* purified by heating at 60°C in 10*N* NaOH for 24 h. Cool resin and rinse 10 times each with alternating portions of 1*N* HCl, metal-free water, 1*N* NaOH, and metal-free water.

g. Metal-free seawater (or brine): Fill a 1.4-cm-ID × 20-cm-long borosilicate glass column to within 2 cm of the top with purified chelating resin. Elute resin with successive 50-mL portions of 1*N* HCl, metal-free water, 1*N* NaOH, and metal-free water at the rate of 5 mL/min just before use. Pass salt water or brine through the column at a rate of 5 mL/min to extract trace metals present. Discard the first 10 bed volumes (300 mL) of eluate.

4. Procedures

a. Sample pretreatment: Before analysis, pretreat all samples as indicated below. Rinse all glassware with 1 + 1 HNO_3 and water. Carry out digestion procedures in a clean, dust-free laboratory area to avoid sample contamination. For digestion of trace aluminum, use polypropylene or TFE utensils to avoid leachable aluminum from glassware.

1) Dissolved metals—See Section 3030B. For samples requiring arsenic and/or selenium analysis add 3 mL 30% hydrogen peroxide and an appropriate concentration of nickel before analysis. For all other metals no further pretreatment is required except for adding an optional matrix modifier (see Table 3113:I).

2) Total recoverable metals (Al, Sb, Ba, Be, Cd, Cr, Co, Cu, Fe, Pb, Mn, Mo, Ni, Ag, and Sn)—NOTE: Sb and Sn are recovered unless HCl is used in the digestion. See Section 3030D. Quantitatively transfer digested sample to a 100-mL volumetric flask, add an appropriate amount of matrix modifier (if necessary, see Table 3113:I), and dilute to volume with water.

3) Total recoverable metals (As, Se)—Transfer 100 mL of shaken sample, 1 mL conc HNO_3, and 2 mL 30% H_2O_2 to a clean, acid-washed 250-mL beaker. Heat on a hot plate without allowing solution to boil until volume has been reduced to about 50 mL. Remove from hot plate and let cool to room temperature. Add an appropriate concentration of nickel (See Table 3113:I), and dilute to volume in a 100-mL volumetric flask with water. Simultaneously prepare a digested blank by substituting water for sample and proceed with digestion as described above.

b. Instrument operation: Mount and align furnace device according to manufacturer's instructions. Turn on instrument and strip-chart recorders. Select appropriate light source and adjust to recommended electrical setting. Select proper wavelength and set all conditions according to manufacturer's instructions, including background correction. Background correction is important when elements are determined at short wavelengths or when sample has a high level of dissolved solids. In general, background correction is usually not necessary at wavelengths longer than 350 nm. Above 350 nm deuterium arc background correction is not useful and other types must be used.

Select proper inert- or sheath-gas flow. In some cases, it is desirable to interrupt the inert-gas flow during atomization. Such interruption results in increased sensitivity by increasing residence time of the atomic vapor in the optical path. Gas interruption also increases background absorption and intensifies interference effects. Consider advantages and disadvantages of this option for each matrix when optimizing analytical conditions.

*Chelex 100, or equivalent, available from Bio-Rad Laboratories, Richmond, Calif.